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Applicant HULSTAERT, Frank et al	

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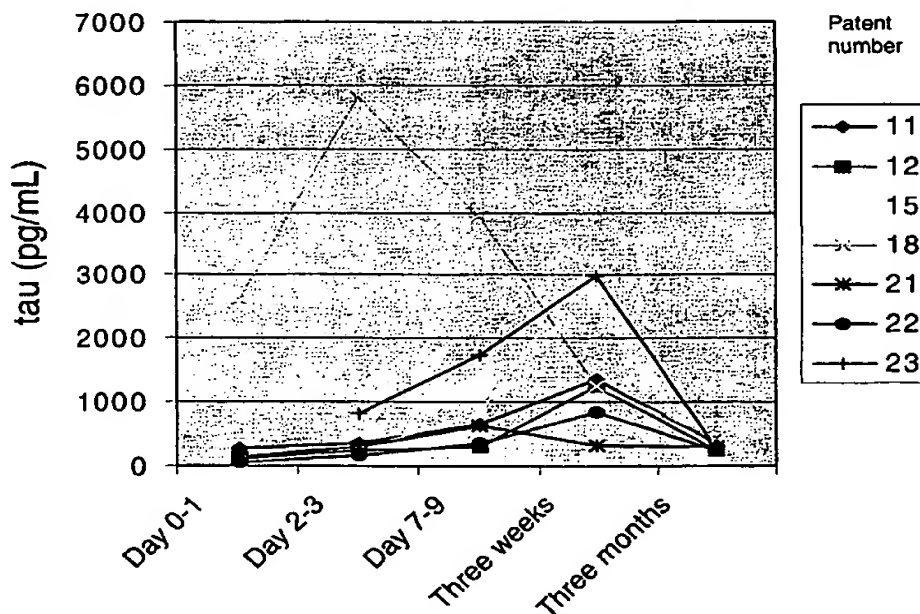
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(54) Title: TAU AS A MARKER FOR EARLY CNS DAMAGE



(57) Abstract

The present invention provides a new method for the early diagnosis of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms. This new method comprises the step of determining and/or quantifying the level of tau in said individual and comparing it to the level of tau in control healthy individuals.

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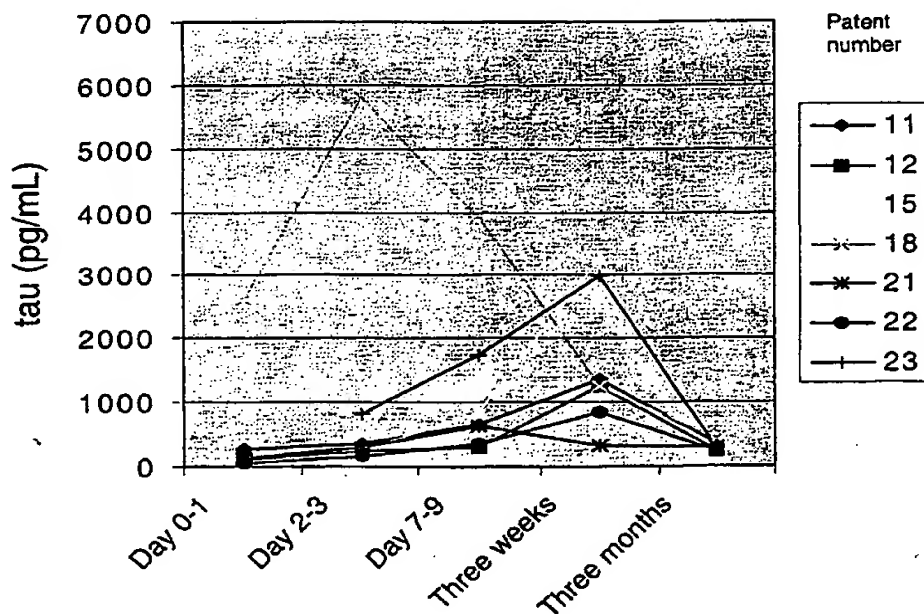
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(54) Title: TAU AS A MARKER FOR EARLY CNS DAMAGE



(57) Abstract

The present invention provides a new method for the early diagnosis of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms. This new method comprises the step of determining and/or quantifying the level of tau in said individual and comparing it to the level of tau in control healthy individuals.

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TAU AS A MARKER FOR EARLY CNS DAMAGE

FIELD OF THE INVENTION

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The present invention relates to the field of CNS damage. The present invention relates to a new method for the early diagnosis of CNS damage by detection and/or quantification of tau.

10 BACKGROUND OF THE INVENTION

Damage of the central nervous system (CNS damage) is caused by various inducing agents among which different disease processes, physical or chemical agents, anoxia and ischemia. Disease processes include space occupying lesions, invasion or metastasis of the brain caused by different malignancies and/or infection by a number of organisms.

15 Tumors of the CNS, may originate locally (primary tumors) or may spread to the CNS (metastases). Primary tumors arise from glial cells (astrocytoma, oligodendroglioma, glioblastoma), ependymal cells (ependymoma) or supporting tissue (meningioma, schwannoma, papilloma of the choroid plexus). In childhood, tumors arise from more primitive cells
20 (medulloblastoma, neuroblastoma, chordoma). Malignant astrocytoma or glioblastoma is the most common type of primary tumor in adults over age 20. Both benign and malignant primary CNS tumors are capable of producing neurologic impairment.

Leukemia is the most common type of cancer in children. During the last twenty years, the survival of children with leukemia has improved markedly based on the routine use of intensive
25 chemotherapy alone or as combined treatment (radiotherapy and chemotherapy). Currently, the estimated overall 10-year survival rate is around 75%. Given the increasing number of childhood leukemia survivors, concern has arisen about long-term effects of anti-cancer chemotherapy and/or radiotherapy resulting in possible damage to the central nervous system and the need for an early quantitative determination of this CNS damage is increasing.

30 Bacterial meningitis may be defined as an inflammation in response to bacterial infection of the pia-arachnoid and the fluid residing in the space which it encloses and also of the fluid in the

ventricles of the brain. The incidence of bacterial meningitis is between 4.6 and 10 cases per 100000 persons per year. *H. influenzae* is the most frequent cause, followed by *N. meningitidis* and *S. pneumoniae*. Once developed, characteristic features of bacterial meningitis include an increase in intracranial pressure, disruption of the blood-brain barrier, cerebral edema, and alterations in cerebral blood flow. The longer the duration of meningitis and the less effective the treatment, the greater the chances that complications and neurologic residua will develop. Approximately 10 percent of infants and children who have bacterial meningitis will be left with persistent unilateral or bilateral sensory hearing loss. Approximately 30 percent of children who have had bacterial meningitis later will turn out to have subtle learning deficits (Wilson et al., 1991).

Viruses can also affect the central nervous system in a variety of ways resulting in a distinction between viral meningitis, viral encephalitis, myelitis and CNS diseases due to slow virus infection. Other conditions that may cause CNS damage are chemical agents such as pharmaceuticals, chemotherapy or exposure to chemical compounds, and physical agents. Head injuries are frequent in industrialised countries, affecting many patients in the prime of life. To appreciate the medical and social magnitude of this problem it needs only to be recognised that almost 10 million Americans have head injuries yearly, about 20 percent serious enough to cause brain damage. Another cause for CNS damage may be anoxia or ischemia. Anoxic-ischemic encephalopathy is a common and often disastrous condition, caused by a lack of oxygen to the brain, resulting from hypotension or respiratory failure. Acute ischemic stroke causes neuronal damage and is a major cause of neurological handicap in western society. Perinatal asphyxia may be associated with CNS damage as well. To date, clinical, electroencephalographic and neuroradiologic evaluation, together with cerebral blood flow studies are the most readily available methods. However, early and accurate evaluation of the severity of brain damage after a hypoxic-ischemic event, remains one of the most difficult problems in neonatal care.

For the detection of CNS invasion in leukemic children current diagnostic procedures include lumbar puncture, eye fundoscopy and brain imaging (Raichle, 1998). However, these diagnostic methods only allow detection of the CNS damage in a more advanced stage while already ongoing CNS damage in the early stages may be missed by these methods. Therefore, there is a need for additional diagnostic methods that allow early detection of CNS damage.

A number of neurological markers have recently become available which reflect conditions of the central nervous system, relating to cell death, axon growth/re-induction, inflammation and/or blood-brain barrier dysfunction. The microtubule-associated protein tau exists in different isoforms, of which 4 to 6 are found in adult brain but only 1 isoform is detected in fetal brain. The diversity of the isoforms is generated from a single gene on human chromosome 17 by alternative mRNA splicing (Himmler, 1989; Goedert et al., 1989; Andreadis et al., 1992). The most striking feature of tau protein, as deduced from molecular cloning, is a stretch of 31 or 32 amino acids, occurring in the carboxy-terminal part of the molecule, which can be repeated either 3 or 4 times. Additional diversity is generated through 29 or 58 amino acid-long insertions in the NH₂-terminal part of tau molecules (Goedert et al., 1989). In vivo tau promotes microtubule assembly and stability in the axonal compartment of neurons by interactions involving its microtubule binding domain which is localised in the repeat region of tau (255-381) (Lewis et al., 1988). In normal circumstances adult brain contains 2 - 3 mol phosphate per mole of tau (Selden and Pollard, 1983; Ksiezak-Reding et al., 1992). Phosphorylation of different sites in normal tau as studied in rat and humans is dependent on the developmental state (Lee et al., 1991; Bramblett et al., 1993; Goedert et al., 1993). Tau variants of 60, 64 and 68 kDa arising as a consequence of phosphorylation have been detected in brain areas showing neurofibrillary tangles (Delacourte et al., 1990; Goedert et al., 1992; Flament and Delacourte, 1990; Greenberg and Davies, 1990). These brains contain 6-8 mol phosphate per mol tau (Ksiezak-Reding et al., 1992). In tau isolated from paired helical filaments, phosphorylation can occur at several positions (Iqbal et al., 1989; Lee et al., 1991; Hasegawa et al., 1992). Detection of normally and abnormally phosphorylated tau in brain extracts is done either via antibodies (Mab Alz50: Ghanbari et al., 1990; Mab Ab423: Harrington et al., 1991; Mab AT120 : Vandermeeren et al., 1993; Mab AT180; Mab AT270 : International application published under WO 95/17429 and Mab AT8 : International application published under WO 93/08302), or via the change in molecular weight (Flament and Delacourte, 1990), or else by functional assay (Bramblett et al., 1992). A combination of monoclonal antibodies, each recognising specific epitopes of tau, has been used to detect the presence of normally and abnormally phosphorylated tau in CSF (Van de Voorde et al., 1995). Tau has been used as a marker to discriminate dementia with altered cytoskeletal properties such as Alzheimer's disease from normal aged subjects or from patients with other types of dementia.

AIMS OF THE INVENTION

It is an aim of the present invention to provide a method for the early detection and/or
5 quantification of CNS damage in an individual, said CNS damage being caused by space-
occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or
ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms.

It is a more specific aim of the present invention to provide a method for the early detection
and/or quantification of CNS damage in an individual, said CNS damage being caused a primary
10 brain tumour, benign or malignant, brain metastasis or a subdural haematoma.

It is another more specific aim of the present invention to provide a method for the early detection
and/or quantification of CNS damage in an individual, said CNS damage being caused by invasion
of the CNS by leukemia, lymphoma or breast cancer.

It is another more specific aim of the present invention to provide a method for the early detection
15 and/or quantification of CNS damage in an individual, said CNS damage being caused by bacteria
or viruses causing encephalitis or meningitis.

It is another more specific aim of the present invention to provide a method for the early detection
and/or quantification of CNS damage in an individual, said CNS damage being caused by stroke,
by cerebral infarction, by cerebral haemorrhage, by thrombosis, by perinatal asphyxia, by
20 Binswanger disease or by vasculitis.

It is another more specific aim of the present invention to provide a method for the early detection
and/or quantification of CNS damage in an individual, said CNS damage being caused by
chemotherapy.

It is another more specific aim of the present invention to provide a method for the early detection
25 and/or quantification of CNS damage in an individual, said CNS damage being caused by trauma,
stroke, intracranial pressure or radiation.

It is another aim of the present invention to provide a method for the early detection and/or
quantification of CNS damage in an individual, said CNS damage being caused by space-
occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or
30 ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms in
order to evaluate the effect of treatment on said CNS damage.

It is another aim of the present invention to provide a kit for the early diagnosis of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms:

- 5 It is another more specific aim of the present invention to provide a kit for the early diagnosis of CNS damage in an individual, said CNS damage being caused a primary brain tumour, benign or malignant, brain metastasis or a subdural haematoma.

It is another more specific aim of the present invention to provide a kit for the early diagnosis of CNS damage in an individual, said CNS damage being caused by invasion of the CNS by
10 leukemia, lymphoma or breast cancer.

It is another more specific aim of the present invention to provide a kit for the early diagnosis of CNS damage in an individual, said CNS damage being caused by bacteria or viruses causing encephalitis or meningitis.

- It is another more specific aim of the present invention to provide a kit for the early diagnosis of
15 CNS damage in an individual, said CNS damage being caused by stroke, by cerebral infarction, by cerebral haemorrhage, by thrombosis, by perinatal asphyxia, by Binswanger disease or by vasculitis.

It is another more specific aim of the present invention to provide a kit for the early diagnosis of CNS damage in an individual, said CNS damage being caused by chemotherapy.

- 20 It is another more specific aim of the present invention to provide a kit for the early diagnosis of CNS damage in an individual, said CNS damage being caused by trauma, stroke, intracranial pressure or radiation.

It is another aim of the present invention to provide a method to screen or monitor the effect of compounds which prevent or treat CNS damage.

- 25 All the aims of the present invention are considered to be met by the embodiments as set out below.

DETAILED DESCRIPTION OF THE INVENTION

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The present invention relates to a method for the early detection and/or quantification of CNS

damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents or by a combination of these mechanisms. This method comprises the step of determining and/or quantifying the level of tau in an individual and comparing it to the level of tau in control
5 healthy individuals.

The present invention relates to the surprising finding that tau levels in CSF samples from children with leukemia are increased compared to upper limit values for healthy individuals. These increased tau levels are an indication of latent central nervous system invasion and already on-
10 going CNS damage long before this CNS damage can be measured by the current diagnostic procedures. Also in individuals that were suffering space occupying lesions of the CNS, invasion or metastasis of the CNS, ischemia, stroke or meningitis, increased tau levels were observed in an early stage. Accordingly, tau can be used as an aspecific marker for the early detection of CNS damage caused by invasion of the CNS by leukemia and in general, as an aspecific marker for the early detection of CNS damage caused by CNS damaging agents such as space-occupying lesion
15 of the CNS, invasion or metastasis of the CNS, organisms, anoxia or ischemia, chemical agents, physical agents, or a combination of these mechanisms.

The central nervous system (CNS) is that part of the nervous system which, in vertebrates, consists of the brain and spinal cord, to which sensory impulses are transmitted and from which motor impulses pass out, and which supervises and co-ordinates the activity of the entire nervous
20 system.

The term "CNS damage" refers to any condition of the CNS which is associated with a neuronal malfunctioning and which is caused by a specific inducing agent or damaging agent. More specifically, CNS damage refers to disease processes that include but are not limited to space-occupying lesions of the CNS, invasion or metastasis of the CNS and/or organisms. Space-
25 occupying lesions may be, for example, primary brain tumours, benign or malignant, brain metastasis, parasite derived cysts such as *Taenia solium* or *Echinococcus granulosus*, hydrocephalus and/or subdural haematoma. Invasion or metastasis of the CNS can be caused by malignancies such as leukemia, lymphoma, breast cancer, lung cancer, melanoma and/or gastrointestinal malignancies or other types of cancers. Organisms that may infect the CNS and cause
30 CNS damage include but are not limited to prions, viruses, bacteria or parasites. Bacteria and viruses that infect the CNS may cause meningitis, encephalitis, neuro aids or neuroborreliose.

They include but are not limited to *Neisseria meningitidis*, *H. influenzae*, *S. pneumoniae*, *Herpes simplex meningoencephalitis* and *Herpes simplex gluteolis*. CNS damage can also be caused by anoxia or ischemia during anesthesia, perinatal asphyxia, drowning, asthma, stroke, cerebral infarction, thrombosis, cerebral haemorrhage, CO poisoning, Binswanger disease and/or vasculitis.

5 Chemical agents causing CNS damage include but are not limited to gene therapy, pharmaceuticals, chemotherapy and exposure to chemical compounds. CNS damage can also be caused by physical agents such as trauma, radiation, hypothermia, hyperthermia, intracranial pressure or stroke. It is also possible that more than one of the above mentioned causing agents are responsible for the CNS damage.

10 The present invention thus provides a method for the early detection and/or quantification of said CNS damage by determining the level of tau. "Early detection and/or quantification of CNS damage" means that the CNS damage is determined by a method that allows it to be detected before it is detectable by the current methods.

The term "tau" as referred to in the present application can be any form of tau, including any state
15 of phosphorylation. The level of tau is determined qualitatively and/or quantitatively as a measure for the degree of CNS damage. Tau can be detected in vitro as well as in vivo.

The method for the early in vitro detection of CNS damage in an individual comprises the steps of obtaining a sample from said individual, determining and/or quantifying the level of tau in said sample and comparing it to the level of tau in a sample of control healthy individuals. The term
20 "sample" refers to any source of biological material, for instance body fluids, hair, epithelial cells, peripheral blood or any other sample comprising tau protein. In a preferred embodiment, tau can be detected and/or quantified in vitro by analysis of the level of tau in a body fluid sample of the patient. The term "body fluid" refers to all fluids that are present in the human body including but not limited to blood, lymph, urine and cerebrospinal fluid (CSF). In a more specific embodiment
25 of the present invention tau is detected and/or quantified in a cerebrospinal fluid sample taken from the patient. In another specific embodiment of the invention tau is detected and/or quantified in a sample of blood derivatives of the patient. The blood sample can include the whole sample as taken from the patient. More preferably the blood sample includes a plasma sample or a serum sample.

30 Tau can be detected and/or quantified by any method known, including but not limited to the use of antibodies, the change in molecular weight (Flament and Delacourte, 1990), or else by

functional assay (Bramblett et al., 1992). In a preferred embodiment tau can be detected by an immunoassay comprising at least the following steps:

- obtaining a sample from the patient; and
- bringing said sample into contact with a monoclonal antibody (primary antibody or capturing antibody) recognising tau, under conditions being suitable for producing an antigen-antibody complex; and
- detecting the immunological binding of said antibody to said sample.

Advantageously, the monoclonal antibody used in the invention is in an immobilised state on a suitable support. Alternatively, the present process may be put into practice by using any other

immunoassay format known to the person skilled in the art.

The process for the detection of the antigen can then be carried out by bringing together said antigen-antibody complex formed by the antigen and the primary antibody recognising tau with:

a) a secondary antibody (or detector antibody)

*which can be a monoclonal antibody recognising an epitope of the tau-primary antibody complex but not recognising the primary antibody alone, or

*which can be a polyclonal antibody recognising an epitope of the tau-primary antibody complex but not recognising the primary antibody alone, with said polyclonal antibody being preferably purified by immunoaffinity chromatography using immobilised tau or the tau-primary antibody complex.

b) a marker either for specific tagging or coupling with said secondary antibody, with said marker being any possible marker known to the person skilled in the art;

c) appropriate buffer solutions for carrying out the immunological reaction between the primary antibody and the sample, between the secondary antibody and the tau-primary antibody complex and/or between the bound secondary antibody and the marker, and,

d) possibly also, for standardisation purposes, a purified protein or synthetic peptide reactive with the antibodies that recognise tau.

Advantageously, the secondary antibody itself carries a marker or a group for direct or indirect coupling with a marker.

The term "epitope" refers to that portion of the antigen-antibody complex that is specifically bound by an antibody-combining site. Epitopes may be determined by any of the techniques known in the art or may be predicted by a variety of computer prediction models known in the

art.

The expression "recognising", "reacting with", "immunological binding" or "producing an antigen-antibody complex" as used in the present invention is to be interpreted that binding between the antigen and antibody occurs under all conditions that respect the immunological properties of the antibody and the antigen.

Any monoclonal or polyclonal antibody that specifically recognises tau may be used for the detection of tau. Antibodies specifically recognising normally and/or abnormally phosphorylated tau include Alz50 (Ghanbari et al., 1990), Ab423 (Harrington et al., 1991), AT8 (International application published under WO 93/08302), AT120 (Vandermeeren et al., 1993); AT180 and AT270 (International application published under WO 95/17429) and AT100 (International application published under WO 96/04309). But also other antibodies known in the art that specifically recognise tau can be used.

The method for the early in vitro detection and/or quantification of CNS damage in an individual can also be used to evaluate the effect of a certain treatment on the CNS damage in said individual. Possible treatments that might influence the status of the CNS include but are not limited to drug treatments, chemotherapy, physical therapy, including radiotherapy and gene therapy.

The method for the early in vivo detection and/or quantification of CNS damage in an individual comprises the steps of determining and/or quantifying the level of tau in said individual and comparing it to the level of tau in control healthy individuals. In a preferred embodiment, tau can be detected in vivo by in vivo imaging. Tau can be visualised in situ by non-invasive methods including but not limited to brain imaging methods described by Arbit et al. (1995), Tamada et al. (1995), Wakabayashi et al. (1995), Huang et al. (1996), Sandroock et al. (1996), Mariani et al. (1997). These in vivo imaging methods may allow the localisation and visualisation of tau, for example, by use of labelled antibodies recognising tau.

Tau can also be used as an aspecific marker for in vivo imaging to evaluate the effect of a certain treatment on the CNS damage in an individual. Possible treatments that might influence the status of the CNS include but are not limited to drug treatments, chemotherapy, physical therapy, including radiotherapy and gene therapy.

The present invention further relates to the use of tau as an aspecific marker for the manufacture of a diagnostic kit for the early detection in an individual of CNS damage caused by space-

occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms.

The present invention further relates to the use of tau as an aspecific marker for the manufacture of a diagnostic kit for the early detection in an individual of CNS damage caused by a primary
5 brain tumour, benign or malignant, brain metastasis, or a subdural haematoma.

The present invention further relates to the use of tau as an aspecific marker for the manufacture of a diagnostic kit for the early detection in an individual of CNS damage caused by invasion or metastasis of the CNS by leukemia, lymphoma or breast cancer.

The present invention further relates to the use of tau as an aspecific marker for the manufacture
10 of a diagnostic kit for the early detection in an individual of CNS damage caused by bacteria or viruses.

The present invention further relates to the use of tau as an aspecific marker for the manufacture of a diagnostic kit for the early detection in an individual of CNS damage caused by stroke, by cerebral infarction, by thrombosis, by cerebral haemorrhage, by perinatal asphyxia, by Binswanger
15 disease or by vasculitis.

The present invention further relates to the use of tau as an aspecific marker for the manufacture of a diagnostic kit for the early detection in an individual of CNS damage caused by chemotherapy.

The present invention further relates to the use of tau as an aspecific marker for the manufacture
20 of a diagnostic kit for the early detection in an individual of CNS damage caused by trauma, stroke, intracranial pressure or radiation.

The present invention further relates to a kit for the in vitro or in vivo diagnosis in an individual of CNS damage caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a
25 combination of these mechanisms. Any kit that provides a tool for the detection of tau can be used for the diagnosis of the above-mentioned CNS damage.

A preferred kit for the in vitro diagnosis in an individual of CNS damage caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms is
30 based on an immunoassay and comprises:

- at least a monoclonal antibody (primary antibody) which forms an immunological

complex with an epitope of the tau protein;

- a secondary antibody

* which can be a monoclonal antibody recognising an epitope of the tau-primary antibody complex but not recognising the primary antibody alone, or

5 * which can be a polyclonal antibody recognising an epitope of the tau-primary antibody complex but not recognising the primary antibody alone, with said polyclonal antibody being preferably purified by immunoaffinity chromatography using immobilised tau protein or immobilised tau-primary antibody complex;

10 - a marker either for specific tagging or coupling with said secondary antibody;

- appropriate buffer solutions for carrying out the immunological reaction between the primary antibody and a test sample, between the secondary antibody and the tau-primary antibody complex, and/or between the bound secondary antibody and the marker;

15 - possibly, for standardisation purposes, a purified protein or synthetic peptide containing one of more tau epitopes.

The present invention also relates to a method to screen or monitor the effect of compounds which prevent or treat CNS damage comprising the step of determining the level of tau and comparing it to the level of tau in a control sample.

20 The present invention will now be illustrated by reference to the following examples that set forth particularly advantageous embodiments. However, it should be noted that these examples are illustrative and can not be construed as to restrict the invention in any way.

Table 1: Treatment Protocol for patients with non-B-cell ALL/NHL (EORTC 58881).

Treatment phase	Drugs	Dose	Route	Days
Prephase	Prednisolone	60 mg/m ² /d	PO	day 1 - 7
	MTX	according to age	IT	day 1 (<u>LP1</u>), day 8 (<u>LP2</u>), day 22 (<u>LP3</u>)
Protocol I: induction (37 days)	Prednisolone	60 mg/m ² /d	PO	day 8 - 28
		20 mg/m ² /d	PO	day 29 - 31
		10 mg/m ² /d	PO	day 32 - 34
		5 g/m ² /d	PO	day 35 - 37
	Vincristine	1.5 mg/m ²	IV	day 8, 15, 22, 29
	Daunorubicine	30 mg/m ²	IV	day 8, 15, 22, 29
Protocol I: consolidation (26 days)	E coli asparaginase	10000 U/m ²	IV	day 12, 15, 19, 22, 25, 29, 32, 35
	Cyclophosphamide	1000 mg/m ²	IV	day 36, 63
	6-mercaptopurine	60 mg/m ²	PO	day 36 - 63
	Ara-C	75 mg/m ²	IV	day 38 - 41, 45 - 48, 52 - 55, 59 - 62
	MTX	according to age	IT	day 38 (<u>LP4</u>), 52 (<u>LP5</u>)
(14 days)				

Table 1. Continued.

Treatment phase	Drugs	Dose	Route	Days
Interval therapy (56 days)	6-mercaptopurine	25 mg/m ²	PO	day 1 - 56
	MTX	5000 mg/m ²	IV	day 8, 22, 36, 50
	MTX	according to age	IT	day 9 (<u>LP6</u>), 23 (<u>LP7</u>), 37 (<u>LP8</u>), 51 (<u>LP9</u>)
(14 days)				
Protocol II: induction (35 days)	Dexamethasone	6 mg/m ² /d	PO	day 1 - 21
		3 mg/m ² /d	PO	day 22 - 35
		1 mg/m ² /d	PO	day 26 - 29
	Vincristine	1.5 mg/m ²	IV	day 8, 15, 22, 29
	Adriamycin	30 mg/m ²	IV	day 8, 15, 22, 29
	E coli asparaginase	10000 U/m ²	IV	day 8, 11, 15, 18
Protocol II: consolidation (14 days)	Cyclophosphamide	1000 mg/m ²	IV	day 36
	6-thioguanine	60 mg/m ²	PO	day 36 - 49
	Ara-C	75 mg/m ²	IV	day 38 - 41, 45 - 48
	MTX	according to age	IT	day 38 (<u>LP10</u>)
(14 days)				
Maintenance	6-mercaptopurine	50 mg/m ² /d	PO	Total duration of treatment = 2 years
	MTX	20 mg/m ² weekly	PO	

Table 2. Treatment protocol for patients with B-cell NHL (UKCCSG 9602).

Treatment phase	Drugs	Dose	Route	Days
COP (7 Days)	Prednisolone	60 mg/m ² /d	PO	day 1 - 7
	Vincristine	1 mg/m ²	IV	day 1
	Cyclophosphamide	300 mg/m ²	IV	day 1
	MTX	according to age	IT	day 1 (<u>LP1</u>)
	Hydrocortisone	according to age	IT	day 1 (<u>LP1</u>)
*** Start depending on the status of the patient				
COPADM 1 and COPADM 2 (5 days)	Prednisolone	60 mg/m ² /d	PO	day 1 - 5
	Vincristine	2 mg/m ²	IV	day 1
	Cyclophosphamide	500 mg/m ² /d	IV	day 2 - 4
	Doxorubicine	60 mg/m ²	IV	day 2
	MTX	according to age	IT	day 2 (<u>LP2</u>), 6 (<u>LP3</u>)
		3000 mg/m ²	IV	day 1
	Hydrocortisone	according to age	IT	day 2 (<u>LP2</u>), 6 (<u>LP3</u>)
*** Start depending on the status of the patient				

Table 2. Continued.

Treatment phase	Drugs	Dose	Route	Days
CYM 1 and CYM 2 (6 days)	MTX	according to age	IT	day 2 (<u>LP4</u>)
		3000 mg/m ²	IV	day 1
	Hydrocortisone	according to age	IT	day 2 (<u>LP4</u>), 7 (<u>LP5</u>)
	Ara-C	according to age	IT	day 7 (<u>LP5</u>)
		100 mg/m ² /d	IV	day 2-6
*** Start depending on the status of the patient				
COPADM 3 (5 days)	Prednisolone	60 mg/m ² /d	PO	day 1 - 5
	Vincristine	2 mg/m ²	IV	day 1
	Cyclophosphamide	500 mg/m ² /d	IV	day 2 - 3
	Doxorubicine	60 mg/m ²	IV	day 2
	MTX	according to age	IT	day 2
		3000 mg/m ²	IV	day 1
	Hydrocortisone	according to age	IT	day 2

IT = intra thecal; IV = intravenous, PO = per os, LP = lumbar puncture, MTX = methotrexate, Ara-C = arabinoside

Table 3: Treatment Protocol for patients with AML (EORTC 58921).

Treatment phase	Drugs	Dose	Route	Days
Induction	Ara-C	100 mg/m ²	IV	day 1, 2
	Ara-C	200 mg/m ² /d	IV	day 3 - 8
	Mitoxantrone	10 mg/m ² /d	IV	day 3 - 5
	VP16	150 mg/m ² /d	IV	day 6 - 8
	Ara-C	according to age	IT	day 1 (<u>LP1</u>), 8 (<u>LP2</u>)
First intensification	Ara-C	3000 mg/m ² /d	IV	day 1 - 4
	Mitoxantrone	10 mg/m ² /d	IV	day 5 - 7
	Daunorubicin	20 mg/m ² /d	IV	day 1 - 4
Second intensification	Ara-C	200 mg/m ² /d	IV	day 1 - 4
	VP16	100 mg/m ² /d	IV	day 1 - 4
	6-thioguanine	100 mg/m ² /d	PO	day 1 - 4
	Dexamethasone	6 mg/m ² /d	PO	day 1 - 4
	Ara-C	according to age	IT	day 1 (<u>LP3</u>), 4 (<u>LP4</u>)
Third intensification	Ara-C	2000 mg/m ² /d	IV	day 1 - 3
	VP16	125 mg/m ² /d	IV	day 2 - 5
Maintenance	6-thioguanine	40 mg/m ²	PO	1 year
	Ara-C	40 mg/m ²	SC	4 days/month

IT = intra thecal; IV = intravenous, PO = per os, SC = subcutaneous, LP = lumbar puncture, Ara-C = arabinoside, VP16 =

Table 4. Level of tau in CSF samples of patients with possible CNS damage caused by different factors.

Cause of CNS damage^c	Centre^a	Patient	Age	Sex^b	Tau level (pg/ml)
SPACE OCCUPYING LESIONS, INVASION OR METASTASIS					
Subdural haematoma	01	025	39	M	263
Anaplastic oligoastrocytoma	01	032	68	M	329
Cerebral metastasis	08	024	70	F	292
Oligodendroglioma WHO III	08	031	48	M	335
Metastasis of breast cancer	08	020	52	F	150
BLEEDING, INFARCTION OR ISCHEMIA					
Stroke - CVA	01	021	62	F	451
Multiple lacunar stroke	01	026	56	F	250
Stroke - CVA	04	004	50	F	105
Acute but limited stroke	05	032	72	M	248
Subarachnoid hemorrhage	08	019	50	M	216
Chronic post-anoxia (vegetative state)	05	051	37	F	446
Binswanger disease	10	014	74	M	347
Vasculitis	10	009	57	F	1250
Cerebral ischemia	10	005	80	M	273
Ischemia	10	006	71	F	574
Superior sagittal sinus thrombosis	01	023	41	M	773

Table 4. Continued.

Cause of CNS damage ^c	Centre ^a	Patient	Age	Sex ^b	Tau level (pg/ml)
ORGANISMS					
Cryptococcal meningitis	05	033	29	M	1143
Neuroborreliose	05	045	16	M	141
Chronic meningitis, unknown cause	05	046	34	F	452
Meningococcal meningitis	05	049	24	M	37
Bacterial meningitis	05	059	73	F	446
Neuro aids	05	060	45	F	237
Pneumococcal meningitis	05	063	70	M	111
Bacterial meningitis	06	026	25	M	1250
Bacterial meningitis	06	027	19	M	37
TBC meningitis	06	028	54	M	720
Bacterial meningitis	06	029	23	M	37
Bacterial meningitis	06	030	28	M	88
Viral meningitis	06	037	82	M	314
Bacterial meningitis	08	018	49	F	205
Viral meningitis	08	029	29	F	170
CONTROLS					
Muscle weakness and hysterical conversion	01	020	39	F	282
Facial palsy (peripheral nerve disease, normal CSF)	05	041	44	M	141
Tension (psychogenic) headache	05	031	49	F	37
Psychogenic headache and neurosis	05	036	56	F	131
Psychogenic signs and symptoms	05	040	48	F	220
Major hysteria	05	042	47	F	471
Lumbar disc prolaps and sciatica	05	047	74	M	195

Table 4. Continued.

Cause of CNS damage ^c	Centre ^a	Patient	Age	Sex ^b	Tau level (pg/ml)
CONTROLS					
Control (associated Sjogren disease)	05	065	52	F	107
Control (neurosis)	05	079	40	F	90
Depression	10	008	59	F	201
Depression	10	010	69	F	139
Myalgia, myositis	04	003	31	M	37
Myalgia, myositis	04	005	35	F	435
Malaise, fatigue	04	006	50	M	98
Neck pain, vertigo	08	011	39	F	192
Headache	08	017	49	M	113
Depression	08	021	57	M	361
Headache, strabismus convergens	08	033	32	F	239
Bell's palsy	06	031	38	M	90
Bell's palsy	06	032	22	F	131
Bell's palsy	06	033	73	F	231
Bell's palsy	06	034	74	F	144
Carpal tunnel syndrome	06	035	71	M	190
Carpal tunnel syndrome	06	036	90	M	665

^a 01: Prof. P. Cras, University Hospital, Neurology, Antwerp, Belgium and Dr. A. Daniels, UIA, Laboratory of Neurobiology, Antwerp, Belgium; 04: Dr. P.D. Mehta, Institute Basic Research, Staten Island, NY, USA; 05: Dr. A. Ivanoiu, UCL St-Luc, Laboratory of Neurochemistry, Brussels, Belgium; 06: Prof. P.P. De Deyn, UIA, Laboratory of Neurochemistry, Antwerp, Belgium; 08: Dr. C. Bancher, Lainz Hospital, Neurology, Vienna, Austria; 10: Dr. J. Wiltfang, Georg-August University, Gerontopsychiatry, Göttingen, Germany.

^b F: female; M: male.

^c In cases where multiple factors may contribute to the damage, only the factor supposed to be

most relevant is given.

5 FIGURE LEGENDS

Figure 1. Tau values at diagnosis, before any treatment was given: 1. Control children; 2. AML; 3. AML-CNS+; 4. CML; 5. MDS; 6. B-NHL; 7. Non-B-ALL; 8. Non-B-ALL CNS+; 9. VHR non-B-ALL.

10

Figure 2. CSF-tau levels in seven patients after acute ischemic stroke. The CSF samples were collected at income (day 0-1), day 2-3, day 7-8, day 21-22 (3 weeks) and day 90-110 (3 months).

Figure 3. CSF-tau levels at the time of maximal release in relation to the size of the infarction as measured by CT scan.

15

EXAMPLES

20 Example 1: Increased tau levels in children with leukemia

To evaluate the influence of chemotherapy on neuronal damage, a longitudinal study was conducted, involving 65 children with leukemia (aged 2 to 16 years) without measurable central nervous system involvement, and treated according to standard procedures. A total of 377 CSF samples were analysed. Before each injection, a small volume of fluid was sampled for routine laboratory analysis and the leftover was used in our study. These children were being diagnosed and then treated for their leukemia at the University Hospital of Leuven, Belgium. Tau protein in cerebrospinal fluid was assessed using the INNOTEST hTAU Antigen (Innogenetics, Gent, Belgium).

25
30

For all children suspected to have leukemia, a lumbar puncture was performed before the start of

the treatment to detect the possible presence of leukemic cells, indicative of central nervous system invasion. The tau levels measured at that time, and thus before treatment, would serve as the control level to compare chemotherapy-induced changes in the levels of tau.

- 5 We observed that some children with leukemia already had very high tau levels at diagnosis in spite of the fact that no leukemic cells were detected in the central nervous system. These children constitute a new risk group having brain invasion or leukemia-induced CNS damage, which cannot always be found using current diagnostic procedures (imaging of the brain, lumbar puncture, eye fundoscopy). This was further supported by the increased tau levels seen in one
10 patient having leukemia with proven cellular invasion into the brain (malignant cells in the cerebrospinal fluid).

Example 2: Increased tau levels in patients with leukemia before treatment

15

1. Subjects

Between August 1996 and June 1999, 510 samples of CSF were taken from 82 children being treated for cancer at the Pediatric Hemato-oncology Department of the Catholic University of
20 Leuven, Belgium. CSF samples were only taken in the course of scheduled lumbar punctions (LPs) for staging or treatment for malignancy.

Three groups of patients with hematological malignancies were studied. The largest group consisted of 48 patients with non-B-ALL, treated according to the EORTC protocol 58881 (table 1). Of these children, 20 had CD10(+) blasts (or common ALL), from whom two patients had
25 also Down syndrome (DS) and 1 patient had the Brachmann-de Lange syndrome; 6 patients had common B-cell blasts, 2 patients had common T-cell blasts, 2 patients had pro-B-cell blasts, 9 patients had pre-B-cell blasts, and 9 patients had T-cell blasts. Forty-two children had leukemia, 6 patients had non-Hodgkin's lymphoma stage II (1 patient), Stage III (4 patients) or Stage IV (1 patient). One patient had overt CNS involvement (CNS+), defined according to the study
30 protocol with malignant cells in the CSF. Five patients were considered as very high risk (VHR) patients according to the criteria defined in the protocol (2 patients with t(9;22) and 3 patients

with corticoid-resistance). As the first part of the induction treatment was similar to the other patients, they were included in the analyses according to the induction chemotherapy. Twenty-eight of the patients within non-B-ALL could be followed longitudinally during their treatment period.

- 5 A second group of patients included 10 B-cell non-Hodgkin's lymphoma patients, treated according to the United Kingdom Children Cancers group (UKCCSG 9602) NHL protocol (table 2). Five patients had B-cell lymphomas, 3 patients had Burkitt's lymphoma, and 2 patients had anaplastic large cell lymphoma (ALCL). All patients were treated with the same protocol, except one patient who had B-cell leukemia. Six of these patients were studied longitudinally.
- 10 A third patient group consisted of 9 children with Acute Myeloid Leukemia - myelodysplasia (AML - MDS), of which 2 patients had CNS involvement and two patients had Down syndrome. There were 3 patients with M0, and 1 patient each with M1, M2, M5a or M7 phenotype. Two patients had MDS, of which one had developed AML and was treated with chemotherapy. All these patients, except one MDS patient, were treated according to the EORTC 58921 protocol
- 15 (table 3) and 7 patients were followed longitudinally.

The other patients consisted of a heterogeneous group of children ($n = 9$) in which for clinical reasons an LP was performed. This group includes 3 children with medulloblastoma (staging), 2 children with rhabdomyosarcoma (staging), 1 child with Langerhans cell histiocytosis (LCH, staging), ganglioglioma (staging), germinoma (staging), and retinoblastoma with CNS metastasis

20 (staging and follow up). The control group consisted of 4 children from whom CSF was taken as part of the routine control for possible viral or bacterial infections, but with negative results. One patient with a localized retinoblastoma (staging) and one screened for familial hemophagocytic lymphohistiocytosis (HLH) were also included in the control group. The nearest relatives of the patients gave oral informed consent for participation in the study.

25

2. Methods

Liquor sampling. Lumbar punctures were performed just prior to the IT administration of therapeutics. Five ml of CSF was collected in different polypropylene tubes. One sample was

30 centrifuged immediately at 1500 rpm for 2 minutes to eliminate cells and other insoluble material. The supernatant was stored at -70°C for subsequent analysis. The number of freeze/thaw cycles

was restricted to a minimum.

Measurement of tau in the CSF. All biochemical analyses for the detection of CSF-tau were made without knowledge of the clinical diagnosis. Potentially confounding factors, such as the number of freeze/thaw cycles, recipient type, and volume of sample per assay were standardized for the whole study protocol. CSF-tau levels were determined using a sandwich ELISA (INNOTEST hTAU Antigen, Innogenetics, Gent, Belgium), that measured total tau (both normal and hyperphosphorylated tau). Samples were analyzed firstly alone, at the time of each LP. Afterwards, all samples derived from one patient were analyzed again on one immunoplate. The correlation coefficient between the results from the first and the second approach for a set of 104 samples was 0.901 (95%CI: 0.856 - 0.933). The increases of CSF-tau described in the paper were excluded to be general increases of proteins in the liquor.

3. Results

Normal upper limit levels for tau were firstly determined on CSF samples from the 6 control children. The mean CSF-tau value was 106.2 pg/ml (95%CI = 34.3-178.0). Arbitrary cut-off normal value was considered as 312 pg/ml (mean + 3 SD), which is in the range of values observed in adults (Hulstaert et al., 1999). Moreover, we did not find a correlation between the CSF-tau values at diagnosis and the age of the children (Pearson $r = -0.161$, $CI_{95\%} = -0.3914 - 0.08836$, $n = 64$), suggesting that age has no influence on CSF-tau levels. Clearly pathological CSF-tau values can be considered as above 500 pg/ml.

Tau levels at diagnosis were analyzed for each subgroup of patients (Fig. 1). There was no obvious difference for the tau levels in patients with and without Dow's syndrome (not shown). The two patients with overt CNS invasion (CNS+) had levels of CSF-tau at diagnosis above 312 pg/ml. However, the 2 children with MDS, 7/28 children with non-B-ALL, 1/4 non-B-ALL patients with very high risk criteria, 1/5 patients with AML, and 2/8 patients with B-cell NHL had a level of tau above 312 pg/ml, while using classical diagnostic procedures, CNS invasion was not detected. Three patients with risen intracranial pressure (medulloblastoma), and one patient with germinoma, from which CSF was taken for staging, had high CSF-tau concentrations

(823, 1397, 1500 and 442 pg/ml), in contrast to one patient with grade I astrocytoma who had a normal CSF-tau level (97 pg/ml). One patient with LCH had a normal CSF-tau level of 112 pg/ml. Two patients with rhabdomyosarcoma could be analyzed at diagnosis: one patient with stage I disease had a CSF-tau level of 279 pg/ml, the other patient with stage IV disease had a level of 320 pg/ml. One patient with retinoblastoma and CNS involvement had a CSF-tau level of 1800 pg/ml.

For 33 patients with non-B-ALL, CSF-tau levels at diagnosis did not correlate with tumor burden, as reflected by the white blood cell count (Pearson $r = 0.04575$, CD95%: -0.3024 - 0.3831) or serum LDH (Pearson $r = -0.03002$, CI95%: -0.3696 - 0.3166). In patients with B-NHL, there was no significant correlation between the LDH level and CSF-tau (Pearson $r = -0.3723$, CD95%: -0.8532 - 0.4507).

Example 3: Use CSF-tau as a marker for early detection of possible CNS damage caused by stroke

1. Subject

Seven patients, 3 men and 4 women, 63-81 years old (mean SD, 70.7 ± 7.2 years) with cerebral infarctions admitted to the Unit of Neurology, Sahlgren's University Hospital, Göteborg, Sweden, were incorporated in the study. All patients were included within 72 h of stroke onset.

2. Methods

CSF samples were collected using lumbar puncture. 12 ml was collected and frozen in 0.5 ml aliquots at -80°C until analyzed. CSF samples were collected at income (day 0-1), day 2-3, day 7-8, day 21-22 (3 weeks) and day 90-110 (3 months). CSF-tau levels were determined using a sandwich ELISA (INNOTEST hTAU Antigen, Innogenetics, Gent, Belgium), that measured total tau (both normal and hyperphosphorylated tau).

The extent of brain damage was examined with computerized tomography (CT) of the brain during the first day of admittance. Clinically the patients were also examined using the modified

Scandinavian Stroke Scale Index (SSI; Scandinavian Stroke Study Group, 1985) at onset of stroke and 3 months later for the degree of disability with the Bartel Index (BI; Mohoney and Barthel, 1965).

5 3. Results

CSF-tau showed a marked increase after acute stroke, with a peak after 1-3 weeks and return to normal after 3 months (Fig 2). There was also a correlation between CSF-tau levels and the size of the infarction as measured by CT scan (Fig 3). These results indicate that CSF-tau reflects neuronal damage and degeneration and the level in the CSF depends on the amount of damaged nerve cells. In this study no correlation was found between the clinical data (SSI or BI) and CSF-tau, probably due to small number of patients.

15 Example 4: Use of tau as a marker for early detection of possible CNS damage caused by different damaging agents

1. Subjects

20 A multicentre study was carried out at 8 European and 2 U.S. university centres involved in CSF research, based on residual CSF archived at the centres for research purposes. CSF samples of patients with a broad range of different neurological conditions were included in order to get a general idea about the specificity of the tau marker changes in a variety of pathologies involving the CNS. Neurological controls consisted of subjects without obvious CNS damage (table 4).

25

The study was conducted in accordance with local clinical research regulations. If required additional local Ethics Committee or Institutional Review Board approval was obtained by the investigator prior to the start of the study.

30 2. Methods

CSF samples were collected using lumbar puncture (LP). Only CSF samples containing less than 500 red blood cells per μ l were included in the study. The CSF samples were centrifuged at 2000 g for 10 minutes within 4 hours after LP and kept frozen without thawing. CSF samples from centre 01 had undergone an additional freeze-thaw cycle before analysis. The concentration of total tau comprising normal tau and paired helical filament-tau was measured at the centres using a sandwich ELISA technique (INNOTEST hTAU-Antigen, Innogenetics N.V.).

3. CSF-tau levels in patients with possible CNS damage compared to CSF-tau levels in neurological control subjects

Tau levels in the CSF samples of patients with possible CNS damage caused by space occupying lesions, by invasion or metastasis, by bleeding, infarction or ischemia or by organisms and tau levels in CSF samples of control subjects are shown in table 5. The group of patients that had possibly contracted CNS damage by space occupying lesions, by invasion or metastasis, by bleeding, by infarction or ischemia, or by organisms showed an overall higher tau level than the neurological control patients ($p = 0.022$, two sided Mann Whitney test).

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USA.

CLAIMS

1. A method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion or metastasis
5 of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents or by a combination of these mechanisms, said method comprising the step of determining the level of tau in said individual and comparing it to the level of tau in control healthy individuals.
2. A method for the early in vitro detection and/or quantification of CNS damage in an individual,
10 said CNS damage being caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents or by a combination of these mechanisms, said method comprising the steps of:
 - obtaining a sample from said individual;
 - determining the level of tau in said sample and comparing it to the level of tau in
15 control healthy individuals.
3. A method according to claim 2 in which the sample is taken from the cerebrospinal fluid of the individual.
- 20 4. A method according to claim 2 in which the sample is taken from the blood derivatives of the individual.
5. A method according to any of claims 1 to 4 in which the space-occupying lesion of the CNS is a primary brain tumor, benign or malignant, brain metastasis, or a subdural haematoma.
25
6. A method according to any of claims 1 to 4 in which the invasion or metastasis of the CNS is by leukemia, lymphoma or breast cancer.
7. A method according to any of claims 1 to 4 in which the organisms are bacteria or viruses
30 causing encephalitis or meningitis.

8. A method according to any of claims 1 to 4 in which the anoxia or ischemia is caused by stroke, by cerebral infarction, by cerebral hemorrhage, by thrombosis, by perinatal asphyxia, by Binswanger disease or by vasculitis.
- 5 9. A method according to any of claims 1 to 4 in which the chemical agent is.
10. A method according to any of claims 1 to 4 in which the physical agent is a trauma, stroke, intracranial pressure or radiation.
- 10 11. A method according to any of claims 1 to 10 in which CNS damage is detected and/or quantified in order to evaluate the effect of a certain treatment on said CNS damage.
12. The use of tau as an aspecific marker for the manufacture of a diagnostic kit for the early detection and/or quantification in an individual of CNS damage caused by space-occupying
15 lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms.
13. The use of tau as an aspecific marker according to claim 12 in any method according to claims 1 to 11.
- 20 14. A kit for the early diagnosis of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms, comprising a tool for the detection of tau.
- 25 15. A kit according to claim 14 for use in any method according to claims 1 to 11.
16. A kit according to claims 13 and/or 14 characterised in that said kit comprises:
- a monoclonal antibody (primary antibody) which forms an immunological complex
30 with an epitope of tau;
 - a secondary antibody

- which can be a monoclonal antibody recognising an epitope of the tau-primary antibody complex, but not recognising the primary antibody alone, or
 - which can be a polyclonal antibody recognising an epitope of the tau-primary antibody complex but not recognising the primary antibody alone, with said polyclonal antibody being preferably purified by immunoaffinity chromatography using immobilized tau or immobilized tau-primary antibody complex;
- a marker either for specific tagging or coupling with said secondary antibody;
 - appropriate buffer solutions for carrying out the immunological reaction between the primary antibody and the test sample, between the secondary antibody and the tau-primary antibody complex and/or between the secondary antibody and the marker;
 - possibly, for standardisation purposes, a purified protein or a synthetic peptide containing one or more tau epitopes.

17. A method to screen or monitor the effect of compounds which prevent or treat CNS damage comprising the step of determining the level of tau and comparing it to the level of tau in a control sample.

Figure 1

528 Rec'd PCT/PTO 09 MAR 2001

2/3

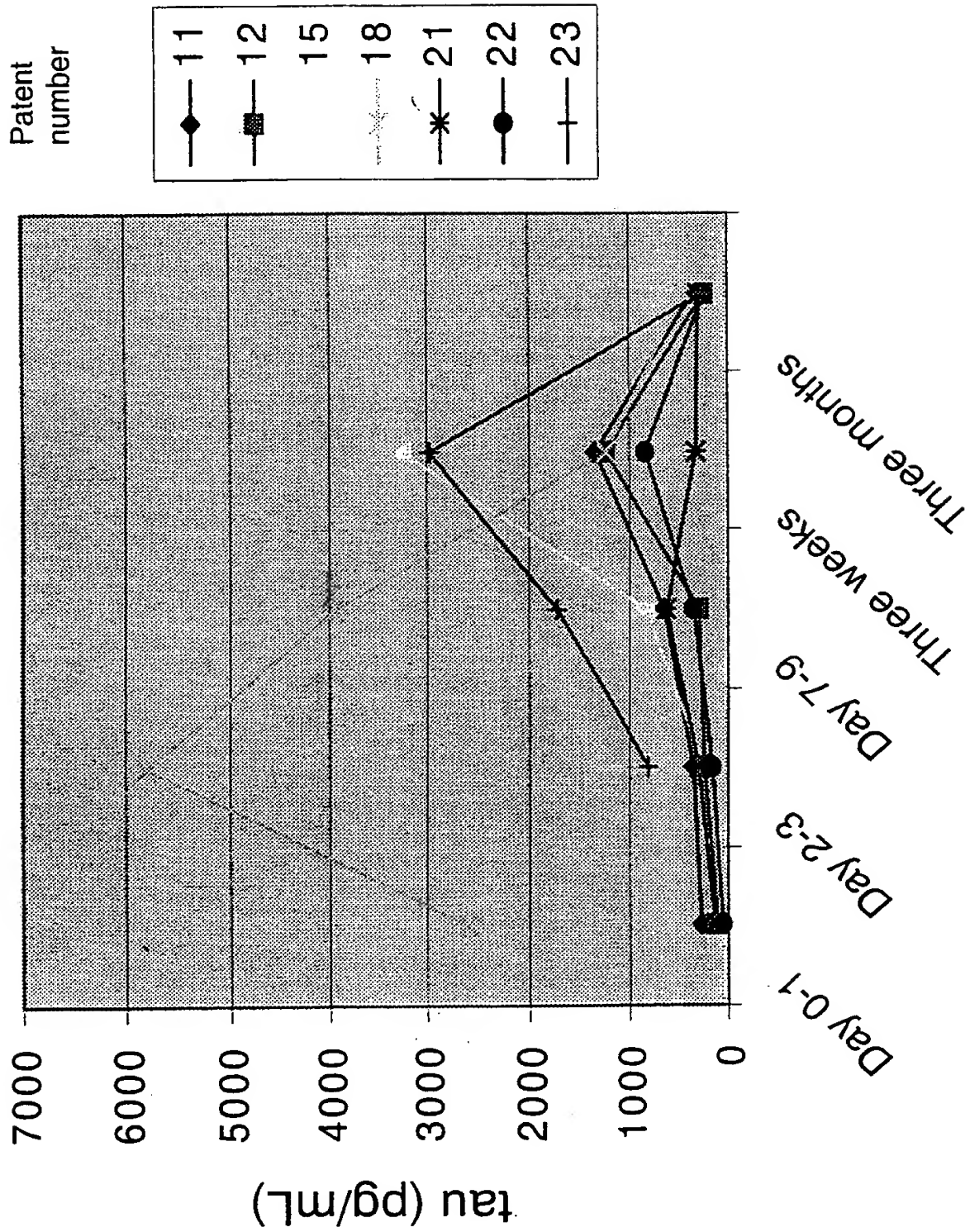


Figure 2

528 Rec'd PCT/PTO 5 MAR 2001

3/3

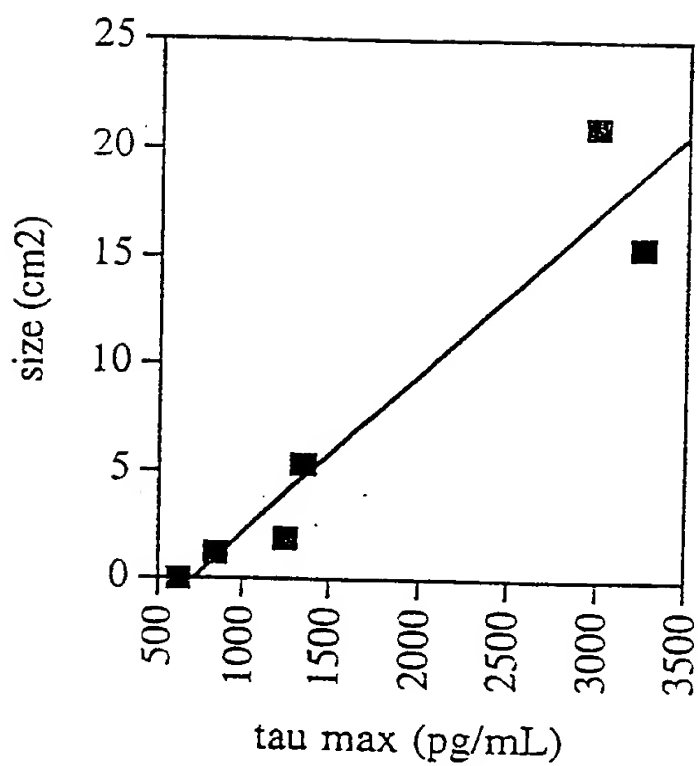


Figure 3

528 Rec'd PCT/P 0 5 MAR 2001

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/06592

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 G01N33/68 G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 13795 A (INNOGENETICS NV ;VANDERMEEREN MARC; MERCKEN MARC; VANMEC) 23 June 1994 (1994-06-23) claims 13,15 ---	1-4,8,9, 12-16
X	SHOJI M ET AL: "Combination assay of CSF Tau, A-beta-1-40 and A-beta-1-42(43) as a biochemical marker of Alzheimer's disease." JOURNAL OF THE NEUROLOGICAL SCIENCES 158 (2). 134-140. ISSN: 0022-510X, 30 June 1998 (1998-06-30), XP002083581 the whole document ---	1-3,9
A	WO 96 04309 A (INNOGENETICS NV ;VANMECHELEN EUGEN (BE); VOORDE ANDRE VAN DE (BE)) 15 February 1996 (1996-02-15) claims 11,14 --- -/--	1-17

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

28 October 1999

Date of mailing of the international search report

09/11/1999

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Gundlach, B

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 99/06592

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>NISHIMURA T ET AL: "Basic and clinical studies on the measurement of tau protein in cerebrospinal fluid as a biological marker for Alzheimer's disease and related disorders: Multicenter study in Japan." METHODS AND FINDINGS IN EXPERIMENTAL AND CLINICAL PHARMACOLOGY 20 (3). 227-235. ISSN: 0379-0355, April 1998 (1998-04), XP002083582 the whole document</p>	1-17
A	<p>TRANCHANT C: "Tau proteins and neurodegenerative diseases" M/S MEDECINE SCIENCES, vol. 13, no. 8/09, August 1997 (1997-08), pages 989-997, XP002083585 the whole document</p>	1-17

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/EP 99/06592

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9413795 A	23-06-1994	AT 165866 T	15-05-1998
		AU 690092 B	23-04-1998
		AU 5809794 A	04-07-1994
		CA 2150816 A	23-06-1994
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WO 9604309 A	15-02-1996	AU 3223495 A	04-03-1996
		CA 2195672 A	15-02-1996
		EP 0772634 A	14-05-1997
		JP 10506381 T	23-06-1998

PATENT COOPERATION TREATY

PCT

REC'D 28 NOV 2000

IPO

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

15

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PCT99.99TAU	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP99/06592	International filing date (day/month/year) 07/09/1999	Priority date (day/month/year) 08/09/1998
International Patent Classification (IPC) or national classification and IPC G01N33/68		
Applicant INNOGENETICS N.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 8 sheets, including this cover sheet.

- ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 10/04/2000	Date of completion of this report 23.11.2000
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Wagner, R Telephone No. +49 89 2399 7357 

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/06592

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:

Description, pages:

1-30 as originally filed

Claims, No.:

1-17 as originally filed

Drawings, sheets:

1/3-3/3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/06592

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1, 9, 12, 13, 17.

because:

☒ the said international application, or the said claims Nos. 1, 17 in regards to industrial applicability relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 9, 12, 13 are so unclear that no meaningful opinion could be formed (*specify*):
see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 5-8, 10, 11, 17

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/06592

	No:	Claims	1-4, 14-16
Inventive step (IS)	Yes:	Claims	5-8, 10
	No:	Claims	1-4, 11, 14-17
Industrial applicability (IA)	Yes:	Claims	2-8, 10-11, 14-16
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

R Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 1 and 17 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).
2. Claim 9 is not complete and therefore not clear (Article 6 PCT).
3. Claims 12 and 13 are not clear (Article 6 PCT)- see section VIII-6.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement.

1. For the assessment of the present claims 1 and 17 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to a method of diagnosis carried out on the human body which is the case for the present claims 1 and 17 which do not exclude that the method can be carried out in vivo. This is supported by the description (page 7, line 16 and page 9 lines 18-25) stating that the method can also be carried out in vivo.
2. Reference is made to the following documents:

D1: WO 94 13795 A (INNOGENETICS NV ;VANDERMEEREN MARC;
MERCKEN MARC; VANMEC) 23 June 1994 (1994-06-23)

D2: SHOJI M ET AL: 'Combination assay of CSF Tau, A-beta-1-40 and A-beta-1-42(43) as a biochemical marker of Alzheimer's disease.' JOURNAL OF THE NEUROLOGICAL SCIENCES 158 (2). 134-140. ISSN: 0022-510X, 30

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP99/06592

June 1998 (1998-06-30), XP002083581

D3: WO 96 04309 A (INNOGENETICS NV ;VANMECHELEN EUGEN (BE);
VOORDE ANDRE VAN DE (BE)) 15 February 1996 (1996-02-15)

3. If the objections of item VIII-5 had been overcome claims 1-4 would be considered as being not novel (Article 33(2) PCT) because D1 (claim 14), D2 (abstract), D3 (page 10, lines 7-22), D4 (abstract) disclose methods for the diagnosis Alzheimer's disease. Alzheimer's disease falls within the large and vague definition of space-occupying lesions because in the case of Alzheimer's disease cerebral space is occupied by senile plaques and neurofibrillar tangles.
4. If the objections of item VIII-5 had been overcome by providing data showing a direct correlation between specific lesions and a difference in tau levels between patients with said specific lesions and healthy subjects, claims 5-8, 10 would be considered as novel (Article 33(2) PCT) and inventive (Article 33(3) PCT) regarding the specific lesions supported by data.
5. If the objections of item VIII-5 had been overcome claim 11 would be considered as novel but its subject-matter would lack an inventive step (Article 33(3) PCT) because it is obvious to use a method for the quantification of lesions to evaluate the effect of a treatment.
6. Claims 12 and 13 are not clear and contrary to Article 6 PCT (see sections III-3 and VIII-6). The applicants attention is drawn to the fact that kits for the detection of tau are not new (Article 33(2) PCT) because D1 (claim 15) and D3 (claim 14) disclose kits which are based on the use of tau as marker for the detection of Alzheimer's disease, which can also be considered as space-occupying lesion (see also V-3).
7. Claim 14 has to be construed as being directed to a kit comprising a "tool for the detection of tau" because the possibility to use for detection of CNS damage is not limiting the scope of claim (GL III-4.8). Claim 14 and dependant claims 15-16 are not novel (Article 33(2) PCT) because D1 (claim 15) and D3 (claim 13) disclose a kit comprising a monoclonal antibody which forms an immunological complex with

an epitope of tau, a second antibody, a marker for tagging or coupling, appropriate buffer solutions and possibly a tau standard.

8. If the objection regarding the lack of support by the description had been overcome, claim 17 would be novel (Article 33(2) PCT) but it is obvious for the skilled person to use the determination of the level of tau which is used as a marker of CNS damage in Alzheimer's disease also to monitor a possible effect of a treatment of Alzheimer's. Therefore the subject-matter of claim 17 lacks an inventive step (Article 33(3) PCT).

Re Item VII

Certain defects in the international application

1. The legend of figure 2 (drawing sheet 2/3) should read "patient number" instead of "patent number".
2. The sentence on page 26, line 13 of the description should refer to table 4 instead of table 5.

Re Item VIII

Certain observations on the international application

1. Claim 1 is not clear (Article 6 PCT) because the term "space-occupying lesion" is not a generally accepted term.
2. Claims 1 and 2 are not clear (Article 6 PCT and GL III-4.5) because the expression "early detection" is too vague.
3. Claim 1 and dependant claims 2, 4-11 are not supported by the description (Article 6 PCT) and not sufficiently disclosed (Article 5 PCT) to enable the skilled person to carry out the methods by determining the tau level in samples other than CSF.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP99/06592

4. Claim 3 is not clear (Article 6 PCT) because the term "blood derivatives" is too vague and would also comprise modified components of blood.
5. The subject-matter of claim 1 and dependant claims 2-10 is not supported by the description (Article 6 PCT) and not sufficiently disclosed (Article 5 PCT). The data shown in the examples of the present application do not show a direct correlation between elevated tau levels and CNS damage because CNS invasion could not be determined by classical methods (description page 23, lines 29-30). Despite the statistical difference shown between the group of patients with possible CNS damage and the control patients of table 4 the overlap between the 2 groups is quite large and the application is not providing a cut-off value which would allow to reliably diagnose CNS damage by an elevated tau level.
6. Claims 12 and 13 are not clear (Article 6 PCT) because the wording "use of tau as an aspecific marker for the manufacture of a diagnostic kit" could mean a) that the protein tau itself is used for the manufacture of a kit (e.g. as a standard) or b) the use of the principle of tau as a marker for the manufacture of a diagnostic kit (which is not a limiting technical feature of a kit). In the light of the description and in view of the possible scope of the claim the latter possibility has to be taken into account for the present examination. In addition the term "aspecific marker" is not clear because in general markers need to have some specificity in order to be used as a diagnostic marker.
7. Claim 14 is not clear (Article 6 PCT) because the expression "tool for the detection of tau" is unclear and too vague.
8. Claim 16 is not clear (Article 6 PCT) because the formulation "A kit according to claims 13 and/or 14" implies that claim 13 is also directed to a kit, which is not the case.
9. Claim 17 is not supported by the description (Article 6 PCT) because the present application does not disclose any data showing that a therapeutic effect of a substance can be detected by determining the level of tau.



INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 99/06592

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68 G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 13795 A (INNOGENETICS NV ; VANDERMEEREN MARC; MERCKEN MARC; VANMEC) 23 June 1994 (1994-06-23) claims 13,15 ---	1-4, 8, 9, 12-16
X	SHOJI M ET AL: "Combination assay of CSF Tau, A-beta-1-40 and A-beta-1-42(43) as a biochemical marker of Alzheimer's disease." JOURNAL OF THE NEUROLOGICAL SCIENCES 158 (2). 134-140. ISSN: 0022-510X, 30 June 1998 (1998-06-30), XP002083581 the whole document ---	1-3, 9
A	WO 96 04309 A (INNOGENETICS NV ; VANMECHELEN EUGEN (BE); VOORDE ANDRE VAN DE (BE)) 15 February 1996 (1996-02-15) claims 11,14 --- -/--	1-17



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

28 October 1999

Date of mailing of the international search report

09/11/1999

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/06592

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>NISHIMURA T ET AL: "Basic and clinical studies on the measurement of tau protein in cerebrospinal fluid as a biological marker for Alzheimer's disease and related disorders: Multicenter study in Japan." METHODS AND FINDINGS IN EXPERIMENTAL AND CLINICAL PHARMACOLOGY 20 (3). 227-235. ISSN: 0379-0355, April 1998 (1998-04), XP002083582 the whole document</p> <p style="text-align: center;">----</p>	1-17
A	<p>TRANCHANT C: "Tau proteins and neurodegenerative diseases" M/S MEDECINE SCIENCES, vol. 13, no. 8/09, August 1997 (1997-08), pages 989-997, XP002083585 the whole document</p> <p style="text-align: center;">-----</p>	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 99/06592

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9413795 A	23-06-1994	AT 165866 T	15-05-1998
		AU 690092 B	23-04-1998
		AU 5809794 A	04-07-1994
		CA 2150816 A	23-06-1994
		DE 69318420 D	10-06-1998
		DE 69318420 T	28-01-1999
		EP 0673418 A	27-09-1995
		ES 2118373 T	16-09-1998
		JP 2879975 B	05-04-1999
		JP 8502898 T	02-04-1996
		US 5843779 A	01-12-1998
		US 5861257 A	19-01-1999
WO 9604309 A	15-02-1996	AU 3223495 A	04-03-1996
		CA 2195672 A	15-02-1996
		EP 0772634 A	14-05-1997
		JP 10506381 T	23-06-1998

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ATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PCT99.99TAU	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/EP 99/ 06592	International filing date (day/month/year) 07/09/1999	(Earliest) Priority Date (day/month/year) 08/09/1998
Applicant INNOGENETICS N.V. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing :



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (see Box II).

4. With regard to the title,



the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

5. With regard to the abstract,



the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.



as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.

2



None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 99/06592

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 G01N33/68 G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 13795 A (INNOGENETICS NV ;VANDERMEEREN MARC; MERCKEN MARC; VANMEC) 23 June 1994 (1994-06-23) claims 13,15	1-4,8,9, 12-16
X	SHOJI M ET AL: "Combination assay of CSF Tau, A-beta-1-40 and A-beta-1-42(43) as a biochemical marker of Alzheimer's disease." JOURNAL OF THE NEUROLOGICAL SCIENCES 158 (2). 134-140. ISSN: 0022-510X, 30 June 1998 (1998-06-30), XP002083581 the whole document	1-3,9
A	WO 96 04309 A (INNOGENETICS NV ;VANMECHELEN EUGEN (BE); VOORDE ANDRE VAN DE (BE)) 15 February 1996 (1996-02-15) claims 11,14	1-17



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

28 October 1999

Date of mailing of the international search report

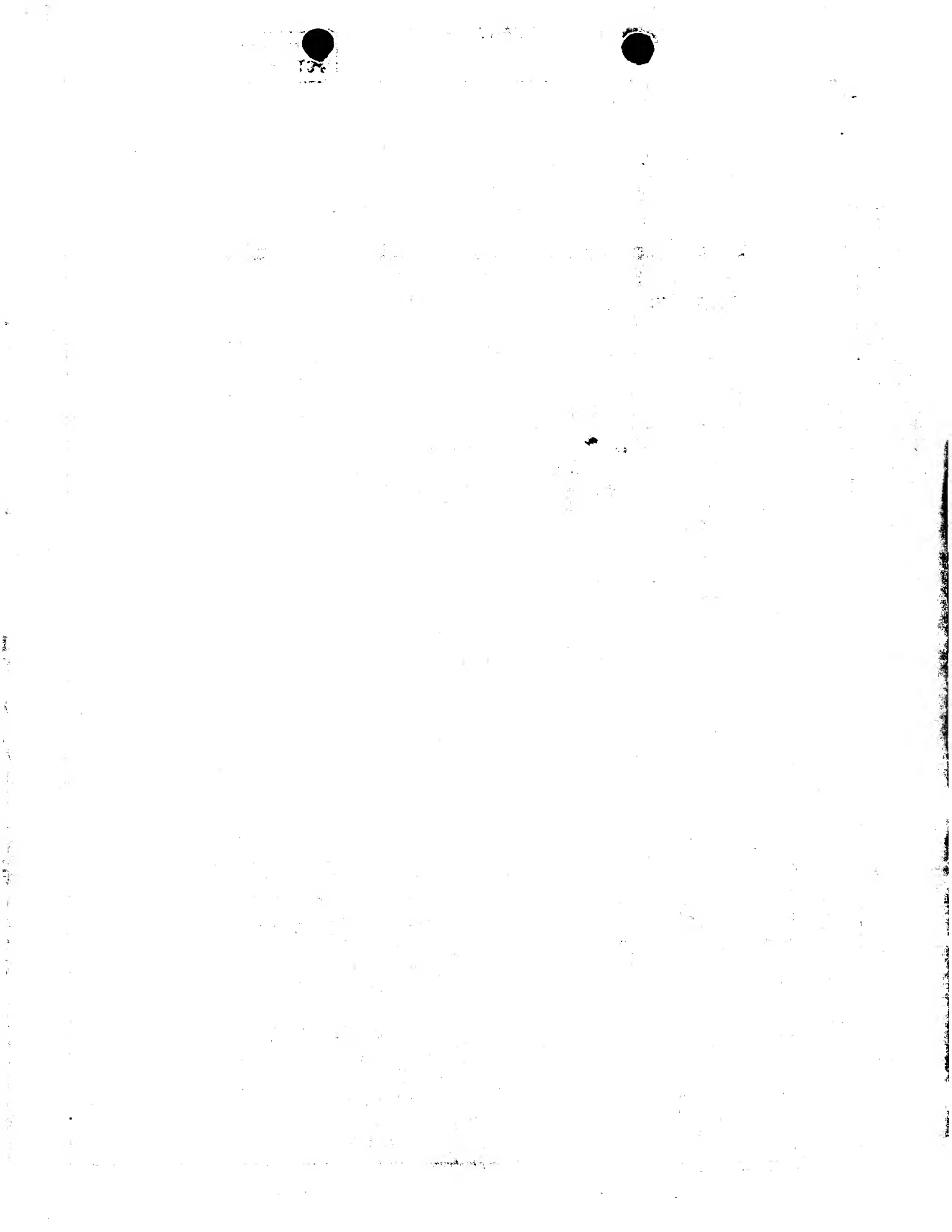
09/11/1999

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Gundlach, B



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